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NUTRITION REVIEWS

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EARLY EXPERIENCES WITH RIBOFLAVIN—A RETROSPECT

It is difficult for one to realize the integrated nature of passing time. Past events appear as more or less independent pictures but not as part of a continuum and it requires much effort to bring them back into life. Scientific progress and its particular way stations are no exceptions. In addition, old recollections have their melancholy undertone since one becomes suddenly aware of the years passed.

My studies leading to the discovery of riboflavin were stimulated by a special interest which I took in pathologic changes of the skin produced in rats by dietary means. Approximately twenty-five years ago, when this work started, the vitamin B-complex was thought to consist of two separate components: (1) vitamin B_1, the antineuritic factor; and (2) vitamin B_2, the antipellagra factor. Claims for the existence of other factors in the complex, named specifically B_3, B_4 and B_5, had been put forward, but owing to unsatisfactory evidence had not been generally accepted.

The British Committee on Accessory Food Factors in 1927 defined vitamin B_2 as "the more heat-stable, water-soluble dietary factor, recently described and named P-P (pellagra-preventive) factor by Goldberger, Wheeler, Lillie and Rogers (1926) and found necessary for maintenance of growth and health and prevention of characteristic skin lesions in rats, and considered by the latter workers to be concerned in the prevention of human pellagra."

For the production of vitamin B_2 deficiency in rats, we first used a ration originally devised by Bourquin and Sherman, which employed an alcoholic extract of wheat as the source of vitamin B_1. Pure vitamin B_1 was at that time not available. On the diet of Bourquin and Sherman, the weight curves of young rats soon flattened out or showed decline. Addition of crude extracts of yeast, rice bran, liver, or of milk concentrates (or whey), in all of which vitamin B_1 was destroyed by autoclaving, restored normal growth.

We then proceeded with the chemical isolation of the active factor, in collaboration with Professor Richard Kuhn and Doctor Th. Wagner-Jauregg. For assay purposes, we used the growth-response in rats fed the experimental ration of Bourquin-Sherman. Modern research workers, accustomed to microbiologic tests which give an answer in twenty-four to forty hours and enable the simultaneous assays of scores of test substances, should be impressed by the fact that each assay for vitamin B_2 in rats required a testing period of three to four weeks, with a corresponding number of prepared experimental animals. Fortunately, however, luck was with us. Doctor Wagner-Jauregg first noted that all the concentrates which proved to be active in the animal assays were colored with an intensity in direct proportion to their biologic effect. The color of these concentrates was green-yellow and fluorescence was distinctly visible in those prepared in neutral solutions or in solutions not strongly acid or alkaline.

This lead proved to be of decisive value in our effort to isolate the active principle. Colored, clear concentrates were exposed to complementary wave lengths of visible light for from six to twenty-four hours. By the use of special filters, care was taken to exclude the ultraviolet spectrum. It was thus definitely established that exposure to visible light destroyed the vitamin B_2 activity of these concentrates which, when

they were not irradiated, were highly effective.

In view of the fact that the biologic activity of concentrates which were nonfluorescent as a result of their preparation in strongly acid or alkaline solution was also destroyed by visible light, it was thought that the inactivation was due probably to light absorption by the colored, dyelike vitamin itself rather than to photosensitization. Such a working hypothesis, which identified vitamin B_2 with a yellow-green pigment, soon met serious difficulties when concentrates that were further purified and more highly colored proved to be biologically inactive. Here the biologist, or animal experimentalist, came to the rescue of the chemist. It was shown that by supplementing the diet with a yeast concentrate from which all colored material had been removed by adsorption, the biologic activity of the colored preparation was restored. Thus, it was proved that vitamin B_2 is not a single substance but that it may be separated into two components, one of which was characteristically a pigment. For me as a nutritionist, this successful recognition of the complex nature of vitamin B_2 offered one of the rare thrills of scientific endeavor, a real reward for many disappointments and failures.

The isolation and identification of the colored component of the B_2-complex were readily achieved. The isolation, first from milk, was accomplished early in 1933 in collaboration with Kuhn and Wagner-Jauregg. Shortly after the isolation of the pigment from milk, similar crystalline preparations were obtained from egg white and liver.

First, the suggestion was made that these yellow-fluorescent, water-soluble pigments be named "flavins" and that a prefix be attached to indicate their origin. Accordingly, the term "lactoflavin," still widely used in Germany, was introduced to designate the flavin isolated from milk, "ovoflavin" from egg white and "hepatoflavin" from liver.

Parallel to this preparatory work, the chemical identification of the flavins was being attacked successfully in different laboratories. Karrer, Kuhn and Stern and their respective collaborators laid the groundwork for the structural analysis.

The first conclusion drawn from the chemical analysis was that all biologically active flavins are chemically identical. The biologically active flavin was recognized as a derivative of isoalloxazine with two methyl groups and a sugar (pentose) radical attached. Since the sugar radical is ribose, the originally proposed term "lactoflavin" has been changed to riboflavin, a designation which in 1937 was adopted by the Council on Pharmacy and Chemistry of the American Medical Association and is now in general use. The terms "lactoflavin," "ovoflavin" and "hepatoflavin" have been discarded.

We may now return to our early experiences with riboflavin. The final experiment proving the growth-promoting activity of the yellow-fluorescent pigment isolated from milk was carried out with the pure substance. Crystalline vitamin B_1 had now become available. Therefore, we replaced the Bourquin-Sherman experimental diet by a semisynthetic ration devoid of vitamin B and supplemented it with crystalline vitamin B_1 in place of the alcoholic wheat extract, employed in the diet of Bourquin-Sherman, with its variable and largely uncontrolled content of B_1 and other constituents of the vitamin B-complex. The weight curve of rats failing to grow on this diet showed no response when pure riboflavin was added to the diet. The further addition of a yeast preparation free from the flavin pigment, such as was obtained according to Peters and his collaborators by adsorption of yeast concentrates on charcoal and subsequent elution with alcohol acidified with hydrochloric acid, resulted in rapid growth of the rats. The yeast

eluate was completely inactive in the absence of riboflavin and later proved to be an excellent source of vitamin B_6, another member of the vitamin B_2-complex.

Lack of riboflavin manifests itself in rats first by retardation and later by complete cessation of growth. The skin manifestations have not been found to be very striking or specific and are rather of a more trivial nature with very definite seborrheic qualities. They are certainly not characteristic of a pellagra-like disease.

The question arose whether riboflavin, which to some extent fell under the definition of vitamin B_2 as given above, had any relation to human pellagra, or to its experimental counterpart, canine blacktongue. In cooperation with Birch and L. J. Harris, we were able to show, independent of and simultaneously with the Wisconsin group under Elvehjem, that riboflavin was different from the specific pellagra-preventive factor (P-P) of Goldberger and his associates. Elvehjem and his colleagues later identified nicotinic acid (amide) with the antipellagra factor, adding another chapter to the fascinating story of the unraveling of the vitamin B_2-complex. Inasmuch as riboflavin was the first member of the vitamin B_2-complex isolated and identified, it is not surprising that it is still often called vitamin B_2, without reference to the comprehensive character of the original term B_2.

While our own research on the possible vitamin nature of yellow-fluorescent pigments, occurring in many tissues and secretions (milk), was progressing, Szent-Györgyi and Warburg with their respective collaborators were studying similar yellow-fluorescent tissue extracts as parts of various enzyme systems. In particular, the papers published by Warburg and Christian in 1932 contained sufficiently revealing chemical data as to make the close relation of their yellow pigment as contained in their so-called "yellow enzymes" to our vitamin-like yellow-fluorescent compound very probable. The only crystalline pure substance which Warburg and Christian obtained from their preparations of "yellow enzymes" was a chloroform-soluble irradiation product. Transformation of riboflavin after irradiation in alkaline solution into this chloroform-soluble degradation product called lumiflavin was also observed by us. However, this compound was found to be biologically inactive. Irradiation of acid or neutral solutions of riboflavin brought about the formation of a blue-fluorescent, biologically equally inactive degradation product, lumichrome, together with varying amounts of lumiflavin.

The identification of the colored component of the "yellow oxidation enzyme" of Warburg and Christian with a vitamin was made possible by the isolation and chemical identification of riboflavin as the final outcome of our own studies carried out in collaboration with R. Kuhn and Th. Wagner-Jauregg.

Among enzymes, riboflavin acts as the prosthetic group of protein-carriers. We introduced the terms "flavoprotein" for compounds in which riboflavin is bound with protein, and "pro-enzyme" for riboflavin in the free state. The term "pro-enzyme" was chosen to indicate the major biochemical role of riboflavin in cellular metabolism.

Thus, based on the pioneering work of Warburg, we were able to link a vitamin-like substance, such as riboflavin, with specific enzymes and enzymatic activity in the living organism.

In addition to the simultaneous studies of Szent-Györgyi and Warburg and their collaborators, Ellinger and Koschara had also called attention to the presence of a yellow-fluorescent water-soluble substance in various tissues and tissue fluids. They, too, prepared purified concentrates of the substance, the vitamin-like quality of which, however, escaped their observation.

The basic observation on the vitamin-

like nature of riboflavin soon received confirmation from several laboratories (Booher, von Euler, etc.). Fortunately, it was only a few years before the essential nature of riboflavin as an exogenous food constituent for the human organism was clearly proven in the studies of Sebrell and Butler (1938). Ariboflavinosis is a generally accepted clinical entity, the specificity of which is perhaps less well delineated than that of several other vitamin-deficiency diseases.

In retrospect—the discovery of riboflavin may be considered as a scientific windfall. It opened the way to the unraveling of the truly complex vitamin B_2-complex. Perhaps even more significantly, it bridged the gap between an essential constituent and cell enzymes and cellular metabolism. Today, with the general acceptance of this idea, it is not considered surprising that water-soluble vitamins represent essential parts of enzyme systems.

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BODY COMPOSITION IN NUTRITIONAL EDEMA

The retention of water as edema fluid is one of the well recognized complications of semistarvation. The occurrence of many cases of nutritional edema during and following the last world war has stimulated renewed interest in the pathologic physiology of this condition. The observations of E. M. Widdowson and R. A. McCance in England and the experimental studies of A. Henschel, O. Mickelsen, H. L. Taylor, and A. Keys from this country have been discussed previously (*Nutrition Reviews* **10,** *181 (1952)*).

A recent contribution to this field has appeared from the Nutrition Research Laboratories, Coonoor, South India, by C. Gopalan, P. S. Venkatachalam, and S. G. Srikantia (*Metabolism* **2,** *335 (1953)*). These workers have applied modern physiologic technics in the study of the profound nutritional edema which occurs in that country. The patients selected for study were mostly beggars with far advanced chronic starvation. When available, millet is the main item of food of such individuals. Green vegetables, fats and animal proteins are eaten in very small amounts. Severe edema was present in all subjects at the time of study. Weakness, vague pains in the extremities and intermittent diarrhea were also common complaints. Sinus bradycardia, hypotension and hypothermia were regularly observed during physical examination. Daily urine volume was greatly decreased (200 to 400 ml.) in most patients. Diminution in urine volume had not been reported in many of the patients observed in Europe.

The size of the various compartments of body fluids was determined in a number of subjects. The plasma volume in 14 uncomplicated cases of nutritional edema averaged 2068 ml., which represented 45 ml. per kilogram of body weight. Although these figures are less than normal, an elevation actually existed in the ratio of plasma to total body solids. The extracellular fluid space was measured by the thiocyanate method and was found to be considerably elevated. In the 15 patients studied the average thiocyanate space was 46.3 (range 37.8 to 59.0) per cent of total body weight. In 5 subjects the measurements were repeated after diuresis had occurred and a drop in extracellular fluid had taken place both in absolute volume and in relation to body weight. The edema in these individuals was therefore more severe than that observed by A. Keys *et al.* ("The Biology of Human Starvation," *Univ. of Minnesota*

Press, Minneapolis (1950)) in whose subjects the rise in extracellular fluid was relative rather than absolute.

Total body water was determined by the urea distribution method. Because of the severe edema present a period of six hours was allowed for equilibration after the administration of urea. Total body water was elevated in all cases. The mean value obtained was 83.3 per cent of body weight. The highest result was 94.3, indicating an incredible degree of hydration. With dietary treatment of these individuals there occurred a decrease in both absolute and relative amounts of body water.

The weights of cellular solids, fat and bone minerals were estimated by the methods of R. A. McCance and E. M. Widdowson (*Proc. Roy. Soc. (London) B* **136,** *115 (1951)*). Because these calculations depend upon a normal degree of cellular hydration they could not be performed directly during the edematous stage. From the figures obtained after the disappearance of clinical edema the weight of these body constituents was estimated using the following assumptions: (1) The weight of body fat depots during the edematous period was considered negligible. In justifying this assumption the authors state that depot fat was not found at autopsy in fatal cases of nutritional edema. The extent of the depletion of fat depots in these cases would have to be much more severe than was observed by McCance and Widdowson in returning German prisoners. (2) The weight of bone minerals during the edematous stage was considered to be equal to the bone mineral weight in the nonedematous period less the calcium and phosphorus ingested during the period. Obviously not all calcium and phosphorus ingested is incorporated into bone so that this represents a maximal correction. Nevertheless the calculated weights of bone minerals during the edematous and nonedematous periods did not differ greatly. (3) The weight of total cellular solids was considered to be the difference between the weight of total body solids and the weight of bone minerals.

In the 5 individuals on whom these calculations were performed the average total body solids was 9.0 kg. (range 4.3 to 14.8 kg.), a figure which is markedly decreased from normal. Likewise the weight of cellular solids represented an abnormally small percentage of total body weight, from 0.75 to 16 per cent. On the basis of cellular solids the degree of intracellular hydration was found to average 87.9 per cent. With the methods used, the normal cellular hydration is found to be about 67 per cent. The authors are cautious in interpreting this remarkable degree of intracellular edema and admit the need for confirmation using more direct measurements of cellular water.

Following dietary treatment and the disappearance of edema the volume of intracellular water decreased in 4 out of 5 patients despite a great increase in cellular solids. The degree of cellular hydration of these patients in the nonedematous stage is given as 67 per cent in all cases. Although the authors do not so state, it would appear that this is an assumed rather than a determined value.

The changes in plasma electrolytes which occurred in edematous patients have been described in a separate publication (S. G. Srikantia, P. S. Venkatachalam, and C. Gopalan, *Metabolism* **2,** *521 (1953)*). Patients suffering from nutritional edema were found to have a slightly low serum sodium (mean 136.3 mEq per liter), a low serum chloride (mean 87.6 mEq per liter) and a slightly elevated serum potassium level (5.2 mEq per liter). Following diuresis induced by a high protein diet the serum sodium actually showed a further decrease (mean 131.7 mEq per liter), indicating that sodium had been lost at an inappropriate rate as compared to water. The levels of serum chloride and potassium in the nonedematous stage had returned toward normal.

Six of the edematous patients were given

10 g. of sodium chloride in addition to the 6.2 g. supplied in the diet. In all cases the excretion of sodium chloride rose promptly in the urine. The patients having a relatively normal urine volume were able within a few days to excrete the administered sodium chloride and edema was not aggravated. In the patients with low urine output salt balance was not regained and edema progressed during the period of salt administration.

A low sodium intake was achieved in 6 subjects by the administration of an ammonium, potassium, cation exchange resin. Symptoms suggestive of ammonium ion toxicity developed in one case. In the remaining cases a brief diuresis with weight loss occurred. Improvement was not sustained despite the continued administration of the resin.

In interpreting these observations the authors have concluded that edema is not produced in malnutrition by the renal retention of sodium directly. The presence of hyponatremia (lowered plasma sodium) and slightly increased plasma potassium suggested to the authors that some degree of adrenal cortical insufficiency might be present.

The severity of the malnutrition and edema seen in these patients makes these studies of particular interest. It is apparent that the disturbances of fluid balance are more severe than those found in the malnutrition observed in the western world. The determining factor may well be the duration of the semistarvation rather than the extent of the caloric deficit. The great increase in extracellular fluid volume confirms earlier concepts, but the degree of intracellular edema differs from the reports of others and needs confirmation. The progression of hyponatremia during therapy is a puzzling observation. A study of the excretion of salt-retaining steroids using the method of Q. B. Deming and J. A. Luetscher (*Proc. Soc. Exp. Biol. Med.* **73,** *171 (1950)*) would be of interest.

DEFECT IN TRYPTOPHAN METABOLISM IN CONGENITAL HYPOPLASTIC ANEMIA

The discovery of an inborn error in the metabolism of tryptophan associated with congenital hypoplastic anemia has been announced in a recent report of K. I. Altman and G. Miller of the University of Rochester (*Nature* **172,** *868 (1953)*). This report is of interest because it indicates that the inability to metabolize and utilize completely an essential nutrient may be of importance in the etiology of this disease. The disease was described by L. K. Diamond and K. D. Blackfan (*Am. J. Dis. Child.* **56,** *464 (1938)*). It is relatively infrequent in occurrence but is first manifest in early infancy, leading to the concept that it is congenital in origin. The characteristic manifestation is an anemia, which is normocytic and normochromic in type, and which has proven to be totally unresponsive to usual hematopoietic stimulants, such as liver extract, iron, folacin and vitamin B_{12}. The number of white blood cells and platelets in the blood is not reduced. The hematologic defect seems to be one affecting erythrogenesis with a failure to produce mature reticulocytes or erythrocytes (I. A. B. Cathie, *Arch. Dis. Childhood* **25,** *313 (1950)*). The only therapy available is supportive, with frequent use of transfusions as hemoglobin and red blood counts decrease to dangerously low levels.

Altman and Miller (*loc. cit.*) noted the occurrence of a strongly blue fluorescent substance in the urine of one child with congenital hypoplastic anemia. This material has been isolated from the urine and identified as anthranilic acid. Identification was achieved by chromatographic methods

and by determination of the melting point and absorption spectrum of the isolated material. Subsequent to this initial demonstration anthranilic acid has been found in urine specimens from each of 8 additional children with the disease. In contrast, none has been observed in urine specimens collected from patients with other diseases in a pediatric ward.

Since anthranilic acid is one of the intermediate products in the metabolic breakdown of tryptophan (*Nutrition Reviews* **11,** *278 (1953)*), the effect of the oral administration of added quantities of tryptophan on the amount of anthranilic acid excreted was determined. It is stated that giving 1.6 g. of L-tryptophan resulted in an increase in urinary anthranilic acid, as well as in the appearance in the urine of other intermediary metabolites of tryptophan. The quantities excreted are not recorded and the other intermediates determined are not named.

It has been demonstrated that anthranilic acid is excreted in relatively large quantities when rats deficient in riboflavin are fed tryptophan, an event which does not occur in rats fed a complete diet (C. C. Porter, I. Clark, and R. H. Silber, *Arch. Biochem.* **18,** *339 (1948)*; M. Mason, *J. Biol. Chem.* **201,** *513 (1953)*). Riboflavin is considered, therefore, to be important in the intermediary metabolism of tryptophan, with deficiency of the vitamin resulting in a shunting of the products of tryptophan breakdown into abnormal pathways so that anthranilic acid is excreted. These considerations prompted the investigators to administer large quantities of riboflavin, 100 to 200 mg. per day, for thirty days to one patient with congenital hypoplastic anemia. Administration of the supplement was followed by a decrease in the quantity of anthranilic acid excreted. However, no change was observed in the hematologic status of the patient.

The finding of anthranilic aciduria as an apparently constant characteristic of congenital hypoplastic anemia and the reduction in the quantity of this abnormal metabolite excreted when riboflavin was administered have led the authors to propose that this decrease is associated with an inborn error of tryptophan metabolism. It is not known how the defect may relate to the abnormality in red blood cell formation. It is of interest that urine specimens from other members of the families of these patients were not found to contain anthranilic acid. Additional details of these studies are to be published later and will be received with interest.

Extension of these observations may lead to an understanding of the pathologic physiology of this disease and perhaps even to rational approaches in therapy. Of equal importance, however, is the opportunity to utilize patients with congenital hypoplastic anemia for further investigations of the intermediary metabolism of tryptophan in man. A recent investigation has provided important new information concerning the roles of riboflavin and vitamin B_6 in tryptophan metabolism in the rat (F. Charconnet-Harding, C. E. Dalgliesh, and A. Neuberger, *Biochem. J.* **53,** *513 (1953)*). It was observed that the feeding of tryptophan to riboflavin-deficient animals resulted in the urinary excretion of kynurenine, kynurenic acid, and xanthurenic acid, as well as anthranilic acid. In contrast, rats deficient in vitamin B_6 excreted kynurenine, kynurenic acid, hydroxykynurenine, and xanthurenic acid but no anthranilic acid. Combined deficiencies of riboflavin and vitamin B_6 were associated with excretion of each of the metabolic products of tryptophan except anthranilic acid. This and other evidence support the view that pyridoxal phosphate is the coenzyme for kynureninase. Although the site of action of riboflavin in this metabolic pathway is not definitely established, it is suggested that riboflavin is concerned with a mechanism of phosphorylation in the formation of hydroxykynurenine from kynurenine.

MATERNAL DIET AND FETAL LIVER

While nutrition education programs have long asserted that the quality of the maternal diet strongly influenced the size and development of the fetus, the number of seriously conducted studies documenting this view has been surprisingly small. In a classic report, J. H. Ebbs, F. F. Tisdall, and W. A. Scott (*J. Nutrition* **22,** *515 (1941)*) compared three groups of pregnant women (supplemented, naturally well fed, and poorly fed) and showed that the incidences of abortions, premature births, stillbirths and neonatal deaths were significantly higher in the group on a poor diet. The women on the good diets had healthier babies and suffered fewer complications, including less toxemias, as well as fewer difficulties during labor, delivery and the postpartum period. A number of studies conducted in Britain (*see Nutrition Reviews* **7,** *139 (1949)*) confirmed these results.

In this country B. S. Burke, V. A. Beal, S. B. Kirkwood, and H. C. Stuart (*Am. J. Obstet. Gynec.* **46,** *38 (1943)*; *J. Nutrition* **26,** *569 (1943)*), Burke, V. V. Harding, and Stuart (*J. Pediat.* **23,** *506 (1943)*; *Nutrition Reviews* **1,** *386 (1943)*; **6,** *299 (1948)*), and Burke, S. S. Stevenson, J. Worcester, and Stuart (*J. Nutrition* **38,** *453 (1949)*) extended these findings using more thorough methods of dietary analysis and covering a greater number of nutrients.

While these studies demonstrated the existence of a relationship between the nutrition of the mother and the survival and growth of the fetus, they gave very little information as to the nature of this influence. Additional clinical studies threw little light on these relationships. For example, J. N. Byrn and N. J. Eastman (*Bull. Johns Hopkins Hosp.* **73,** *132 (1943)*) found that concentrations of vitamin A in the blood of mother and fetus were independent of each other. Similarly, the hemoglobin level of the newborn infant does not reflect that of the mother (M. B. Strauss, *J. Clin. Invest.* **12,** *345 (1933)*). By contrast, there is a correlation between maternal and fetal carotene levels (J. M. Lewis, O. Bodansky, M. C. C. Lillienfeld, and H. Schneider, *Am. J. Dis. Child.* **73,** *143 (1947)*), and a similar correlation between ascorbic acid levels in mother and fetus (B. M. Hamil and co-workers, *Ibid.* **74,** *417 (1947)*).

It can be pointed out that the value of blood levels as indicators of nutrient reserves vary with the nutrient considered. Liver nutrient content, for example, is a much better index of the state of nutrition. In his reports of the growth of lambs before and after birth in relation to the level of nutrition, L. R. Wallace (*J. Agr. Sci.* **38,** *93; 243, 367 (1948)*) showed that the weight of the fetal liver in sheep is particularly responsive to maternal diet. Ewes fed grossly deficient diets produced lambs with birth weights as much as 50 per cent lower than those attained on good maternal diets. The liver weights were even more drastically affected. Wallace also observed that a disproportionately great decrease in liver weight occurred on more moderate reduction of the maternal diet.

While the full term human infant weighs only about 7 per cent of its mother's weight (instead of 14 per cent as in the case of the lamb) and takes forty weeks (instead of twenty) to reach its size, it appeared possible that the liver of the human fetus or newborn infant might be an accurate reflection of maternal nutrition. To check this possibility, C. A. Smith, J. Worcester, and B. S. Burke (*Obstetrics and Gynecology* **1,** *46 (1953)*) investigated the weight and nutrient content of over 100 livers of fetuses or newborn infants, obtained in pregnancies terminating in neonatal death or fetal death between the twelfth and forty-third weeks of fetal age.

As the authors themselves have pointed out, use of the liver to assay transplacental nutrition necessarily restricts case material to stillborn fetuses and infants dying soon

after birth. Despite this handicap, Smith, Worcester, and Burke were able to disclose some interesting relationships between maternal dietary intake and liver content and concentration of certain nutrients. The composition and adequacy of the maternal diets were assessed by the Burke method (B. S. Burke, *J. Am. Dietet. Assn.* **23,** *1041 (1947)*). Concentrations of riboflavin, ascorbic acid, vitamin A, thiamine, iron and nitrogen were measured in the fetal (or neonatal) livers. One hundred eleven livers were studied; however, maternal dietary histories were obtained in only 75 of the pregnancies. Thiamine analyses were limited to 26 livers, and iron determinations were done on 61 specimens. The other nutrients were assayed in sufficient number to permit statistical analysis.

Smith, Worcester, and Burke found that the weight of the fetal liver bears a closer relationship to the protein intake of the mother than to her over-all dietary intake. No correlation was found between vitamin A content in the fetal liver and carotene content of the maternal diet. However, the content in the fetal liver was adversely affected by a poor maternal intake of vitamin A. Mothers who took mineral oil regularly during pregnancy also tended to produce fetuses whose livers contained less than a normal amount of vitamin A. A significant association was shown to exist between the content of ascorbic acid in the fetal liver and the maternal dietary intake of this vitamin; the relationship between intake and concentration was less striking. By contrast, the maternal intake of riboflavin showed a higher degree of correlation with concentration than with content in the fetal liver.

Iron analyses were done on only 36 of the 75 cases with dietary histories. In this small group, no relation between the iron content of the fetal liver and maternal dietary intake of iron was observed. Too few thiamine measurements were made to permit correlation with dietary history.

The authors acknowledge that the mean general ratings of the maternal diets of the group under consideration were predominantly poor. This was to have been anticipated in a study which of necessity dealt only with stillbirths and neonatal deaths. Even so, twins, erythroblastic infants and those born to mothers with diabetes, severe heart disease, or severe toxemia were excluded from the series. There were instances of low fetal liver ratings associated with good maternal diets and, less commonly, of high liver ratings with poor maternal diets. This suggested that the diet histories were sometimes faulty or, alternatively, that even a relatively poor maternal nutritional status may permit satisfactory fetal nutrition. Since dietary histories, however detailed, are not infallible, and since fetal growth in the human species is relatively slow compared with that in many other mammals, it would appear that either or both of these two conjectures can be used to explain the deviant results. As the authors point out, human pregnancy offers the fetus extraordinary opportunities of nourishing itself, even from a limited maternal supply. Much more impressive than the exceptions, however, is the general finding that at least one important fetal tissue reflects maternal intake during pregnancy.

SERUM CONCENTRATION AND URINARY EXCRETION OF VITAMIN B_{12}

According to present concepts, the disease pernicious anemia apparently results from deficiency of vitamin B_{12}, which is brought about by defective absorption of the vitamin from the intestinal tract. Experimental observations of many types support the view that the so-called "intrinsic factor" which is present in gastric secretions is active in promoting absorption of the vitamin and is absent in the stomachs of patients with

pernicious anemia (*Nutrition Reviews* **9,** *281 (1951)*; **10,** *219, 229 (1952)*).

The absorption, utilization, and excretion of vitamin B_{12} in man is the subject of a recent investigation by W. G. Unglaub, H. L. Rosenthal, and G. A. Goldsmith (*J. Lab. Clin. Med.* **43,** *143 (1954)*). In these studies the concentration of the vitamin in serum and the quantity excreted in the urine was determined before and following the oral or parenteral administration of vitamin B_{12} to normal subjects or to patients with pernicious anemia or nutritional macrocytic anemia. The method employed was a microbiologic assay utilizing *Lactobacillus leichmanii* (U. D. Register and H. P. Sarett, *Proc. Soc. Exp. Biol. Med.* **77,** *837 (1951)*; Rosenthal and Sarett, *J. Biol. Chem.* **199,** *433 (1952)*).

The concentration of vitamin B_{12} in serum was found to be much higher in normal subjects than in patients with pernicious anemia. The mean value in 31 normal individuals was 0.21 millimicrograms per milliliter, while the average serum concentration in 6 patients with pernicious anemia in relapse was only 0.04 millimicrograms per milliliter. There were comparable low values in serum samples obtained from 2 individuals with nutritional macrocytic anemia. However, the quantity of the vitamin excreted in the urine by patients with pernicious anemia did not differ significantly from that excreted by the normal persons.

Serum levels were determined at intervals after intramuscular injections of from 10 to 100 micrograms of vitamin B_{12}. Characteristically, there was a prompt increase to maximum values in approximately one hour, followed by a rapid fall for three hours and a slower decrease through twenty-four hours. Peak concentrations reached were roughly proportional to the amount of the vitamin administered. Maximum levels and the shape of the curve were similar in patients with pernicious anemia and in normal subjects. There was somewhat less vitamin B_{12} excreted in the urine of the patients than appeared in the urine of normal individuals given the same dose of the vitamin. The percentage of the dose injected which was recovered in the urine during the twenty-four hours immediately following the injection was greater with larger doses. Thirty and 38 per cent was found in the urine of 2 normal persons given 100 micrograms intramuscularly.

Oral administration of vitamin B_{12} was followed by increases in serum content of the vitamin both in normal individuals and in patients with megaloblastic anemia. These changes were usually of low order, when as little as 500 to 1000 micrograms were given. However, 2 patients with pernicious anemia were found to have a moderate to marked increase in serum vitamin B_{12} and suboptimal hematologic response following this dosage. Administration of 3000 micrograms by mouth resulted in increased serum concentrations in 5 of 6 normal subjects. Peak concentrations were found after four to eight hours and were comparable to those recorded after intramuscular injection of 10 to 25 micrograms of the vitamin. An increase of moderate to marked degree in serum values was observed in 5 of 6 patients with pernicious anemia or nutritional macrocytic anemia given 3000 micrograms orally in a single dose. In all of this group of patients there was a satisfactory hematopoietic response. The quantity of vitamin B_{12} which was excreted in the urine of the normal subjects and patients seemed to bear no definite relationship to the maximum serum activity achieved. The amount excreted was similar to that which occurred in the urine after intramuscular injection of from 10 to 50 micrograms of vitamin B_{12}. However, urinary excretion was increased when oral dosage of the vitamin was repeated.

These observations demonstrate that the patient with pernicious anemia is able to

absorb vitamin B_{12} from the intestinal tract when large quantities of the vitamin are administered. Moreover, the serum concentration of the vitamin increases in these patients as it does in normal subjects given similar quantities by injection or by mouth. These findings are consistent with the clinical observations that hematologic response may be induced and maintained in patients with pernicious anemia when large quantities of vitamin B_{12} are administered by mouth (C. C. Ungley, *Brit. Med. J.* **2,** *905 (1950)*; C. L. Conley *et al.*, *J. Lab. Clin. Med.* **38,** *84 (1951)*). Absorption of the vitamin apparently occurs in the absence of intrinsic factor in these patients and is as effective as in normal persons. This might indicate that intrinsic factor is essential to intestinal absorption only when the vitamin is present in very small amounts as in natural foods.

However, the adequacy of an explanation of the etiology of pernicious anemia solely in terms of an absorptive defect for vitamin B_{12} may be questioned in terms of other observations. It has been demonstrated, for instance, that there is deficient absorption of the vitamin labeled with radioactive cobalt in patients following gastrectomy, a defect quantitatively similar to that in pernicious anemia. However, megaloblastic anemia occurs infrequently in such patients (M. E. Swendseid, J. A. Halsted, and R. L. Libby, *Proc. Soc. Exp. Biol. Med.* **83,** *226 (1953)*; *Nutrition Reviews* **12,** *4 (1954)*). These findings would seem to indicate the importance of further studies of the metabolism and utilization of vitamin B_{12}, as well as a search for factors, other than an absorptive defect, which might contribute to the occurrence of megaloblastic anemia.

POTASSIUM AND PARENTERAL PROTEIN ALIMENTATION

The synthesis of protein from amino acids provided in the diet is a complicated process. The work of P. R. Cannon, L. E. Frazier, and R. H. Hughes (*Metabolism* **1,** *49 (1952)*; *Nutrition Reviews* **12,** *75 (1954)*) indicated that the provision of an adequate supply of potassium is an essential requirement for optimal protein synthesis. These workers fed protein-depleted rats a ration containing an adequate supply of calories, vitamins, amino acids and salts and achieved good protein synthesis. The removal of potassium from the salt mixture resulted in poor food consumption and a failure to gain weight. The approximate "minimal daily requirement" of potassium salt needed for good protein repletion was found to be 20 mg. of potassium chloride daily.

The extension of this significant observation to man has recently been reported by P. M. Frost and J. L. Smith (*Metabolism* **2,** *529 (1953)*). The intravenous administration of amino acid mixtures alone is an ineffective form of parenteral protein alimentation. Protein synthesis can be greatly increased by the simultaneous administration of carbohydrate (E. H. Ellison, R. S. McCleery, R. M. Zollinger, and C. T. Case (*Surgery* **26,** *374 (1949)*). The possibility that potassium added to amino acid solutions containing an adequate caloric source might increase protein synthesis has been examined by Frost and Smith.

Completely parenteral alimentation was used for these studies which covered periods of from seven to fifteen days. An adequate supply of calories was provided by the addition of 12.5 per cent glucose and 2.5 per cent ethyl alcohol to protein hydrolysates. Four patients (group 1) were studied during two preoperative days and five postoperative days. The intravenous fluids provided 3100 calories and 28 g. of nitrogen daily. The balance data of one patient are

published. A negative nitrogen balance occurred when the basic solutions contained only 20 mEq or less of potassium daily. It would seem that the provision of 4 mEq of potassium per gram of nitrogen administered was required for maximal protein synthesis.

Solutions providing 3100 calories and 12 g. of nitrogen were given daily for fifteen days to 2 patients (group 2). Sodium chloride losses were replaced. The effect on nitrogen balance of supplementing the basal parenteral feeding with 40 and 80 mEq of potassium was observed. In the single protocol published a negative nitrogen balance on 40 mEq of potassium was converted into a positive nitrogen balance by the intravenous administration of 80 mEq of potassium.

One patient (group 3) received 3200 calories and 16 g. of nitrogen daily for nine days. A constant electrolyte intake of 120 mEq of sodium chloride and 40 mEq of potassium chloride was included in the parenteral solutions daily. Nitrogen balance was slightly positive throughout the study period; a total retention of 13.5 g. of nitrogen occurred.

A parenteral intake of 2800 calories, 16 g. of nitrogen and 120 mEq of sodium chloride was given daily for ten days to 3 patients (group 4). In the balance study published no potassium supplementation was given during the first five days. During this period a net loss from the body of 132.8 mEq of potassium and 10.2 g. of nitrogen occurred. When 80 mEq of potassium chloride was given with the fluids during the subsequent five-day period 46.8 mEq of potassium and 26.5 g. of nitrogen were retained. The authors state, without presentation of data, that when the order of administration of potassium was reversed the balance studies indicated a similar beneficial effect of potassium.

This is an important paper in the field of parenteral nutrition. It is unfortunate, therefore, that the authors have chosen to present only selected protocols. Although the authors state that the protocols selected were representative, a more compelling argument would have resulted if at least summaries of the other studies had been presented. No clinical data are provided concerning the diagnosis and state of nutrition of the patients studied. A word concerning the difficulties and reactions which occurred during the administration of large volumes of hypertonic solution would be welcome to physicians attempting to follow the outlined regimens.

It is possible to summarize the requirements for parenteral protein repletion: (1) a source of amino acid nitrogen containing the proper mixture of essential amino acids, (2) the simultaneous administration of adequate calories in a readily utilizable form —usually carbohydrate, (3) provision of a minimum of 4 mEq of potassium for each gram of amino acid nitrogen. Undoubtedly this list will be extended to include other intracellular electrolytes and vitamins. The authors have concluded that there are "sufficient supportive data to warrant use of potassium when nitrogen and caloric requirements are fulfilled, to increase glycogen deposition in the liver and at the same time enhance nitrogen utilization in depleted patients."

ANTIBIOTICS AS AIDS IN MEAT PRODUCTION

Antibiotics are being actively investigated in agriculture as supplements to food rations of farm animals. In some cases they lead to increased growth rates with more economical use of feeding stuffs. P. R. Moore *et al.* (*J. Biol. Chem.* **165,** *437 (1946)*) noticed as early as 1946 an increased growth of ducks fed streptomycin. Recently W. E. Gerard, D. C. Read, and J. M. Pensack (*J. Agr. Food Chem.* **1,** *784 (1953)*) made a com-

parative evaluation of several of the newer antibiotics in their effect on the growth of the chick and of swine.

Two control lots were used in the studies with chicks, one being fed the basal ration with no antibiotic and the other receiving the basal ration plus 2 g. per ton of Compenamine, a salt of penicillin G. Thus each new antibiotic could be compared more readily with other antibiotics previously evaluated under standardized conditions. A thirty-day test period produced growth increases of 16 to 20 per cent when 2 g. of penicillin were added per ton of feed. Larger amounts up to 50 g. did not increase this improvement. Significantly high chick growth responses were obtained also by feeding bacitracin, erythromycin and an unnamed antibiotic at high concentrations of about 50 g. per ton of feed.

In their experiments on swine Moore and co-workers found that the feeds supplemented with aureomycin-vitamin B_{12}, erythromycin, and penicillin-bacitracin combinations effected a saving of nine to twelve days in producing market-weight hogs. Those receiving erythromycin consumed less protein supplement and more corn than the other groups. Possibly this antibiotic exerts a greater protein-sparing action than other substances tested. Daily gains averaged 12 to 15 per cent higher for swine fed antibiotics. On the basis of feed costs, all groups fed rations supplemented with antibiotics were more economical to raise than the group receiving the control ration. The most economical gain was obtained by use of a fairly low concentration of penicillin-bacitracin combination of 5 pounds per ton of protein supplement. This combination contained 1 g. of penicillin G and 5 g. of bacitracin per pound.

Such experiments as these raise many questions regarding possible accumulation of antibiotics in the meat or certain parts of the animal body, to produce off-flavors or other undesirable results. It was recently reported by R. W. Bray (*J. Agr. Food Chem.* **1,** *129 (1953)*) that to date there has been no evidence that feeding of antibiotics to hogs to increase their growth rate has any effect on the quality of pork. Workers in Wisconsin, Iowa, and Minnesota agree on this point. With the continual development of new antibiotics and feed supplements, constant diligence in searching for such effects seems necessary.

EFFECT OF INSECTICIDES ON FOOD QUALITY

One of the problems that arise when crops are dusted or sprayed for insect control is the possible deleterious contamination of the next succeeding crop grown on the same land that has accumulated these insecticide residues. This question has arisen in the case of peanuts grown following cotton crops that were dusted with benzene hexachloride. It was reported that serious off-flavors could arise in the peanut butter made from such peanuts. The problem has been investigated by H. Reynolds, G. L. Gilpin, and I. Hornstein (*J. Agr. Food Chem.* **1,** *772 (1953)*), who gave considerable attention to the difficulties involved in the use of taste panels. Peanut butter is a difficult food for panels to judge with accuracy. Considerable training of judges was necessary.

Seven samples of peanut butter were made containing amounts of benzene hexachloride varying from 0 to 7 parts per million (p.p.m.). These were used to establish thresholds of detection, etc. Six of 8 samples made from peanuts grown following a benzene hexachloride-treated cotton crop were scored lower than the control. Forty-five per cent of all judgments of such samples described the samples as having typical benzene hexachloride flavors. No

comparable flavors were ascribed to the control sample. Chemical analyses detected this substance in all 8 samples and none in the control; the amounts were well correlated with the quantities employed in the insecticides applied earlier to the fields producing the peanuts.

Comparisons of panel scores with chemical analyses indicate that the off-flavor is not entirely due to the presence of benzene hexachloride itself, but such flavors may be affected by the condition in which it is present (perhaps as degradation products). When this chemical is added directly to the peanut oil, it is not so objectionable as when found in the peanut butter at much lower concentrations as a natural constituent absorbed from the soil. This appears to be a very complicated problem, and one which may have some serious consequences in certain cases of crop rotations.

Toxaphene has been recommended by federal and state agencies for control of insects which damage alfalfa. An important question involves the amounts of this insecticide that may appear in the milk and tissues of cows fed alfalfa that had been protected by such sprays. This was investigated under Utah (arid climate) conditions by G. Q. Bateman and co-workers (*J. Agr. Food Chem.* **1,** *322 (1953)*).

Toxaphene was applied to plots in a 30-acre field of alfalfa at rates of 0, 1, 2, and 4 pounds per acre, with four replications. On the eighth day after spraying the alfalfa was cut, sun-cured, baled, and stored under cover during feeding trials. Eight cows were employed, 2 being fed hay from each spray treatment. Good economical control of alfalfa weevil larvae was obtained on the 2- and 4-pound-per-acre spray levels, the percentage control being 88 and 94, respectively, on the seventh day after application.

Cows maintained their production of milk on treated hay as well as on the control. No effect of toxaphene on gains in weight during the one hundred and twelve-day feeding period could be attributed to the spray on the feed. Milk samples tested weekly contained toxaphene, generally in proportion to the amount applied to the hay fed the respective cows. The highest value found was 29 p.p.m. Thirty-four days after feeding of the treated hay was discontinued, no toxaphene was found in the milk of even the cow fed most toxaphene, indicating rather rapid elimination via milk secretion.

Data on hay and grain consumption and production of milk and butterfat indicate that, under the conditions of this experiment, no clear effect could be attributed to toxaphene. Analysis of liver, muscle, kidney, and kidney fat showed considerable toxaphene concentration (88 and 197 p.p.m.) in the kidney fat from cows fed hay from the 2 and 4 pound treatments. The other tissues contained no toxaphene. Histologic studies showed no changes that could be attributed to toxaphene poisoning nor were any symptoms of such poisoning observed at any time.

These problems will continue to arise with increased application of chemical control methods to agricultural products.

SURVEY OF THE NATION'S CORN CROP

Corn is used in livestock feeding to a greater extent than any other grain. Surveys of the 1946 and 1947 corn crops were made for composition and reported by the Committee on Feed Composition, Agricultural Board, National Research Council. This report was prepared by B. H. Schneider, H. L. Lucas, and K. C. Beeson (*J. Agr. Food Chem.* **1,** *172 (1953)*). The purposes of the first year's survey were to evaluate the effect of hybrid corn varieties on composition, especially the protein content, and to obtain a nation-wide picture of the nutritive constituents of corn as fed to farm animals.

The 1946 season was normal and 1947 was a "soft corn" year.

The nation was divided into ten regions on the basis of rainfall and temperature. Samples were so distributed that the number in a region was proportional to production. Considerable attention was given to proper sampling to make the statistical calculations significant. Samples were of unground corn, harvested for feeding purposes. Subsamples were sent to numerous laboratories for exhaustive analyses for protein, fat, fiber, nitrogen-free extract, numerous minerals, and vitamins. In 1946, 169 samples were analyzed and in 1947 there were 197 samples.

Although of doubtful importance from the point of view of the practical nutritive value of corn, the magnitude and pattern of regional and state differences are of interest from the standpoint of nutrient composition of feeds in general. States were grouped according to protein content, but, in general, regions of similar protein content did not necessarily correspond to climatic regions. Regional differences in fat content were possibly due to variety and color. The corn crop of the southern states in general showed low iron values. For most nutrients, the number of analyses was considered too few to warrant attempts to define regions as completely as was done for protein. The large within-state variation for most nutrients casts doubt on the practical importance of regional differences.

Soil classes were in general only slightly associated with variations in the composition of corn. In both years the fat content of white corn was higher and the iron content lower than in yellow corn. In general the only practical nutrient difference between white and yellow corn is carotene content. Varietal variation was studied only on yellow corn. With the possible exception of fat, differences in nutrient composition among varieties grown in practice are not of practical nutritional importance. This does not imply that nutritionally improved varieties cannot be developed by proper breeding and selection, but only that the more important commercial varieties appear to be genetically rather uniform with respect to factors determining nutritive value. Fluorine and carotene contents are positively correlated with a relatively large number of other nutrients.

The feed industry has observed that the protein content of corn has declined during the years prior to 1946. This conclusion is verified by this study, for the accepted value of the mean protein content of No. 2 corn in 1936 was 9.4 per cent. The 1946 survey gave a value of 8.7 per cent and in 1947 a value of 9.1 per cent was obtained. The present study does not offer any basis for conclusive reasons for this decline.

For most nutrients, it appears that factors of region, state, or variety per se have little to do with the nutrient content of commercial corn. Soil type and localized climatic effects are indicated as reasons for the variability found. Other possible factors are associated with cultural practices, such as fertilization. Relatively small periodic nation-wide surveys such as these appear to be adequate and very desirable, because new varieties and greater use of fertilizers, especially the micronutrient elements, may permit production of crops under new and different ecologic conditions. Different soil and climatic factors combined with new varieties may change materially the composition of the normal plant.

NEW SOURCES OF WORLD FOOD SUPPLIES

The ever-increasing world population has encouraged nutritionists and economists to study possible means to produce a commensurate increase in food supplies. Unicellular photosynthetic organisms such as *Chlorella* have been enthusiastically advo-

cated by numerous investigators as potential food producers (*see Nutrition Reviews* **9,** *347 (1951)*; **11,** *77 (1953)*). Based on production under laboratory conditions the theoretic annual yield has been calculated to be 30 to 60 tons of dry, high-protein *Chlorella* per acre, which would be very high compared to the yields of ordinary food crops. On the basis of the production of 30 tons of dry weight per acre, this would represent a yield of approximately 15 tons of protein and 2 or more tons of fat. Research on *Chlorella* therefore should be continued even though the theoretic costs for maintenance of an acre of culture are presently of a far different order from the cost of an acre of farmland. If the population of the world continues to increase at the present rate, such a course may have to be adopted regardless of cost.

In a series of papers presented at the Nutritional Panel of the British Society of Chemical Industry, new and challenging sources of future food supplies were considered. The potentialities for more efficient use of sunlight for food production were discussed by N. W. Pirie (*Chem. Ind. (London) No. 19, 442 (1953)*). Sunlight is the present source of energy for the production of all food supplies and the green plant is the main means for utilization of the energy.

More economical use of the vegetation now grown was advocated. Only a relatively small portion of plant crops is eaten directly by man—the greater part (chaff, straw, leaves, etc.) is fed to animals, used as animal bedding, or allowed to rot. Conversion of plant crops to animal tissue, although the latter is more delectable, is wasteful because the over-all efficiency of an animal in the transformation of plant material into human food is never greater than 30 per cent, and is usually only 5 to 10 per cent. As food shortages increase, there is a point where economy of such conversion unquestionably will prove too wasteful to be justified on the basis of flavor and food preference. Much vegetation is used for raw material such as textiles, and nutritionists may find it mandatory to compel the production of more synthetic fibers in order to release these fertile areas of the world for production of food crops.

The increase of crops which have the advantage of being available for direct use as human food offer great economical advantages, but difficulties arise since plants, although excellent sources of carbohydrates, fats and vitamins, are generally poor sources of protein with respect to both quantity and quality. The seeds of many legumes are well known to be rich in protein. In the past, insufficient recognition has been given to the fact that the young, vigorously growing leaves of many plants often have 30 to 40 per cent protein and little fiber. Pirie believes that the high protein content of young leaves is of sufficient importance to justify extensive research to develop plant varieties that could stand up to a continuous cropping of these very young leaves. The interesting observation was made that leaves grown in partial shade have a higher protein content, under conditions otherwise the same, than leaves of comparative size, grown in open sunshine. The production of such protein-rich crops would do a great deal to revolutionize the world feeding situation.

The extraction of nutritious materials present in leaf crops which are now considered to be inedible has been studied. The separation of the fluid cell contents of starch, fat, leaf proteins, sugars and other components of low molecular weight has been carried out ón a laboratory scale with varying success for over a century. Several attempts have been made during the last two decades to accomplish this on a commercial scale. The greatest need at present appears to be a machine which is capable of efficiently pressing the nutritious juice from the ground mass of leaves. Details of the

designs of machines used by N. W. Pirie are given in *World Crops* **4**, *374 (1952)*.

The juice, after coagulation, gave a fluid containing sugars, amides, amino acids and salts, and also a coagulum containing proteins, fats and sugars. The fibrous mass contained most of the cellulose, lignins, and pectins and some remaining proteins, starches and fats. The value of this pressed fiber residue as an animal food depends on the extent to which the protein has been removed. When the crop was simply ground and pressed the dried residue generally contained 1.5 to 2.0 per cent nitrogen. Thus it has a feeding value similar to that of good hay or poor dried grass and was more economical to dry than grass.

Initial analyses of the amino acids present in the fluid (J. W. H. Lugg, *Advances in Protein Chemistry* **5**, *229 (1949)*) suggested that the leaf protein would be capable of supplying human requirements. Hence the generally held opinion that vegetable proteins are less valuable nutritionally than animal proteins may be questioned insofar as leaf proteins are concerned. The fact that seed proteins are considerably less satisfactory than animal proteins may be no reason to extend this opinion to leaf proteins. The leaf is an active metabolic site of the plant and in function is more comparable to animal liver than to plant seed. It may be expected that the proteins present in the metabolically active leaf and liver will have a similar amino acid make-up, but the case for leaf protein production and efficiency has yet to be proven by adequate feeding experiments on both man and animals.

A. C. Thaysen (*Chem. Ind. (London) No. 19, 446 (1953)*) outlined the possibilities of the extensive use of microorganisms, especially yeast, to convert inorganic nitrogen into available human and animal foods. Yeast is able to convert 80 per cent of the inorganic nitrogen supplied into protein at an amazing rate. A fully grown steer, weighing approximately half a ton, produces protein at the rate of about 0.9 pounds per day. Half a ton of soya beans planted and harvested under optimum conditions would give a calculated twenty-four-hour protein output of 82 pounds, but if a half ton of yeast were grown under favorable conditions, 51 tons of protein would be synthesized in twenty-four hours. Nutritionally it is the proteins and vitamins that place the microorganisms in a class by themselves as nutritional supplements.

Thaysen emphasized that the role of yeast should be stressed as a supplement to the normal diet. He expressed the belief that "much harm has been done by some writers, for instance in publications emanating from the United Nations, in interpreting results obtained in the study of microbial proteins on test animals, in which the concentrate was given as the sole source of dietary protein, as applying also to the use of yeast as a supplement only for unbalanced foods." Ample evidence is available to show that, far from being harmful as a supplement, yeast added to diets deficient in essential amino acids or vitamins is highly beneficial for both man and animals. The danger arises when supplementation is excessive and the microbial protein is expected to supply an appropriate amount of the methionine requirement which it is incapable of doing. As an answer to those who believe that the high cost of microbial protein makes it economically infeasible, it is significant to note that, in Hawaii, present-day investigators maintain that they have succeeded in overcoming the economic difficulties heretofore encountered in technical yeast production.

The potentialities of synthetic foods to solve world feeding problems has been discussed by P. N. Williams (*Chem. Ind. (London) No. 24, 582 (1953)*), who noted that oleomargarine is the first man-made product to compete strongly with a major

natural food. The production of this product is not a *de novo* synthesis of fat, since margarine is a structural modification of existing natural sources of fat, either animal or vegetable. However, synthesis of edible oil or fat from simple nonfatty materials has been accomplished by (1) the oxidation of hydrocarbons to fatty acids and the esterification of these with glycerol and (2) production of fats or fatty acids by the action of microorganisms, with, if necessary, esterification of the fatty acid with glycerol. Both of these processes have been undertaken on plant scale in Germany. Some German scientists, O. Flössner (*Ernährung* **8,** *89* (*1943*)), H. Kraut, A. Weichser, R. Hügel, and G. Stumpf (*Biochem. Z.* **318,** *472* (*1948*)), and K. Oberdisse (*Z. f. d. ges. exp. Med.* **114,** *60* (*1944*)), stated that the synthetic fat was satisfactory in every way for human consumption, but H. H. Meyer-Döring (*Klin. Wchnschr.* **27,** *113* (*1949*)) has stated that it should be completely condemned as a food.

Numerous microorganisms, when grown on suitable substrates, have been successfully used by European investigators to produce fats. The Swedish report of E. Tornquist and H. Lundin (*Int. Sug. J.* **23,** *123* (*1951*)) described a process involving *Rhodotorula gracilis* as the microorganism and molasses as the source of carbohydrate. The analyses of fats derived from microorganisms reported by A. Hesse (*Advances in Enzymology* **9,** *653* (*1949*)) indicated that these fats have physical and chemical properties closely resembling typical vegetable oils. Further nutritional studies should be made on synthetic fats as a possible answer to world demands.

W. F. J. Cuthbertson (*Chem. Ind. (London) No. 23, 553* (*1953*)) pointed out that the chemical transformation of natural products could lead to the production of vast amounts of food from material which presently has little or no value. Inedible crop residues consist mainly of polysaccharides and lignins; research is needed to find methods to convert these to assimilable carbohydrates or even fats. Already dextrose has been prepared from sawdust on an industrial scale. Research on the flora in the digestive tract of ruminants may offer the clue to the chemical processes involved, as microorganisms within the rumen convert fibrous and woody material into edible protein, fat and carbohydrate.

Coupled with the above search for new sources of human foods, there should be a continued emphasis on good farming procedures, crop rotation, fertilization, disease prevention, insect control, etc. Many of the above suggestions for new food sources sound far less appetizing than the foods to which we are accustomed. Undoubtedly none of these sources will become available suddenly in large quantities. Instead they will probably first be available in small experimental quantities which can be evaluated for their nutritional qualities. On the basis of these facts, appropriate studies can be undertaken to find ways to use them as supplements to existing foods, or to the development of suitable flavors, consistencies, etc., if their qualities are such as to recommend their development as staple dietary items.

CONSTITUTIONAL DIFFERENCES BETWEEN CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

By careful selection, progeny testing, and close inbreeding during the past sixteen years, H. R. Hunt, C. A. Hoppert and their co-workers (*J. Dent. Res.* **23,** *385* (*1944*); **27,** *154, 486, 553* (*1948*)) have developed two strains of albino rats which differ markedly in their susceptibility to tooth decay. Over 8600 caries-resistant and

caries-susceptible specimens have been observed in studies which have revealed that heredity, coarseness of rice particles, age of animal, and use of molar teeth were factors contributing to caries incidence while sex and secretions of the parotid glands were not significant. In chemical studies on the teeth from caries-susceptible and caries-resistant rats, Hoppert and R. L. Shirley (*Ibid.* **29,** *29* (*1950*)) observed that the per cent ash in the incisors, the per cent phosphorus in the ash of the molars and the incisors, and the rate of absorption and removal of radiophosphorus from the teeth after intraperitoneal injections were the same for both strains.

The cariogenic diet used by Hunt and Hoppert in most studies consisted of 66 per cent coarsely ground rice, 30 per cent powdered whole milk, 3 per cent alfalfa meal, and 1 per cent sodium chloride by weight. During the last six generations, the coarsely ground rice was replaced by a more finely ground rice which was equally cariogenic.

R. F. Sognnaes (*J. Am. Dent. Assn.* **37,** *676* (*1948*)) reported that a nutritionally adequate purified diet with high sucrose content produced noticeable caries in rats if fed during tooth development and thereafter. This finding caused W. H. Stewart, C. A. Hoppert, and H. R. Hunt (*J. Dent. Res.* **32,** *210* (*1953*)) to initiate studies to determine whether hereditary differences between the caries-resistant and caries-susceptible strains in their studies were specific for the natural diet used, or whether these differences would persist when the animals were fed a similar purified diet. Their ration consisted of 57 per cent granulated beet sugar, 10 per cent finely ground rice, 18 per cent casein, 2 per cent corn oil, 5 per cent brewers' yeast, 5 per cent alfalfa meal and 3 per cent salt mixture. The rats used were offspring of breeders of the seventeenth resistant generation and the twenty-first susceptible generation; until 25 days of age, all offspring were continued on the natural ration containing finely ground rice. At 25 days of age the litters were divided into four groups and put on the following dietary regimens: (1) caries-susceptible rats fed the sucrose diet, (2) susceptible rats continued on the finely ground rice ration, (3) resistant rats fed the sucrose diet, and (4) resistant rats fed the fine rice diet. The same environmental controls prevailed which had been maintained for fifteen years during the genetic study. At two-week intervals, each rat was observed for carious lesions and "caries time" was recorded as the number of days between the date the animal was put on the experimental diet and the date when the first carious lesion was observed in the lower molars. Rats were killed when carious lesions were first observed.

Striking differences were observed between the susceptible and resistant strains on both diets. Some resistant rats were caries-free at the termination of the experiment at an average age of 500 days. The mean caries time for the susceptible rats fed the sucrose diet was ninety-two days, while the caries time for the susceptible rats on the fine rice diet was fifty-eight days. The caries-resistant rats, when fed the sucrose diet, had a mean caries time of three hundred and sixty-eight days while the average for the resistant rats on the fine rice was three hundred and seventy-two. Thus the susceptible strain fed the sucrose diet developed carious lesions significantly later than those susceptible rats on the rice diet, while the resistant rats on the sucrose diet did not differ significantly from those resistant rats on the fine rice diet. Hence the hereditary difference was not specific for the natural diet but was still striking on a purified diet containing 57 per cent sucrose. Examination of the molar teeth with the binocular microscope showed no evidence that tooth fracturing initiated caries. Small cavities, deep in the sulci, were consistently seen where fracturing by coarse food particles was improbable.

After the above experiment was under way, a group of 11 caries-susceptible rats was placed on a diet in which confectioners' sugar (powdered sucrose containing 3 per cent starch) replaced the 67 per cent beet sugar and rice. The earliest observation of caries was thirty-seven days after the experiment was initiated, and the average was sixty-five and seven-tenths days. The investigators point out the smallness of this group. Yet the trend of the data conflicts with the view that diets lacking hard coarse particles decrease the incidence of caries. Unfortunately the powdered sucrose diet was not fed to the resistant rats.

In further studies the suspicion arose that caries-susceptible animals weighed less and had less hair than the resistant rats. R. S. Clise and H. R. Hunt (*J. Dent. Res.* **32,** *215 (1953)*) conducted an experiment to determine any possible correlation between the growth rate and pilosity and caries resistance. The 212 male and female rats from the twentieth generation of the susceptible strain and sixteenth generation of the resistant strain were weighed at frequent intervals for one year while they were being maintained on the regular cariogenic rice diet. Average weights were based on 35 or more animals. After one year, 100 rats of similar age (25 of each sex and strain) were selected for a comparative study of the hair densities; 84 were between the ages of 386 and 390 days. After each animal was etherized, the hair on its right side was moistened and brushed, and by means of electric clippers the hair was removed between the shoulder and flank. The clipped portion was carefully measured and the area of the trapezoid calculated. After dirt particles were removed, the dried weight was determined; this weight was divided by the area to give the hair density (milligrams of hair per square centimeter of skin).

During the period of rapid growth, the weights and rates of growth in the two strains were the same in both sexes. Differences became statistically significant in either sex at the relatively advanced age of 44 weeks. At this time the susceptible males weighed 18.7 g. less than the resistant males, and the susceptible females weighed 11.3 g. less than the resistant females. A consistently higher coefficient of variation was noted in the weights of the susceptible strain; this might suggest that the latter strain was not as healthy or that its representatives were influenced by some factor which did not affect the resistant animals to the same extent. The animals of the susceptible strain, both male and female, were found to have significantly less hair than the resistant strain. The hair density in the resistant males was 8.18, in resistant females 9.26, while the density in susceptible males was 6.30 and in susceptible females 6.36.

During this study, one examination for caries was conducted to determine if these rats were typical of respective strains. At 104 days of age, 78 per cent of the susceptible rats had advanced caries, while at 155 days of age only 2.7 per cent of the resistant rats had doubtful carious lesions. Thus the difference in weight was greater after the period of caries incidence. This suggests that the lower weights might be an effect of tooth decay rather than a cause and may have been due to a debility accompanying the destruction of the lower molars by dental caries. The latter hypothesis may be weakened by the fact that the cariogenic diet is ground to such fineness that chewing is not necessary. The fact that the susceptible rats had less hair may be of special interest since both teeth and hair are derived from the ectoderm. On the other hand, the reduced hair density, like body weight, may be an end result of extensive destruction of the lower molars.

In another study of the differences between the two strains, an experiment was conducted by E. C. Nakfoor, H. R. Hunt,

and C. A. Hoppert (*J. Dent. Res.* **31,** *143 (1952)*) to compare the resistance to experimentally produced fracturing of the lower molar teeth of caries-susceptible and caries-resistant rats. Since the two strains showed a marked significant difference with respect to artificially induced fracturing, this might suggest that the molars of the susceptible rats were injured more readily during mastication, leading to the earlier onset and more extensive damage of caries. All rats in these studies were fed the natural diet with fine rice until they were killed at approximately 60 days of age. One half of the lower jaw was immediately placed in a vise to study its resistance to fracturing. Ten cusps on each jaw were chosen for breakage tests. A cylindrical aluminum rod, 7 inches long, with blunt point, was dropped vertically through varying distances upon the cusps. The effect of these blows was observed through a binocular microscope at the instant of impact.

The lower molars of caries-resistant rats showed a greater resistance to fracturing by this procedure than those of caries-susceptible rats. The relative weakness of the molars in the susceptible animals was thought to be a possible indication of a structural difference. Observations with the unaided eye and with the binocular microscope suggested that the crevices in the molars from susceptible rats were wider than those in the resistant rats. This was proposed as a possible cause for the greater mechanical weakness of the susceptible teeth. Wider crevices might also result in a greater impaction of food, leading to more bacterial activity and finally caries. Notwithstanding the data obtained, the investigators believed that fracturing was unimportant in initiating dental caries in these rats. Few fractures were observed in the lower molar teeth of 60 day old susceptible rats, although dental caries was present in nearly half the animals.

These studies to explore constitutional differences between strains of rats with divergent caries-susceptibilities are but the beginning in a much needed extensive search to understand why such profound differences in susceptibility occur. From numerous laboratories, reports have come indicating that various strains of hamsters, cotton rats and the common laboratory rat differ widely in caries-susceptibility when maintained under identical circumstances. These differences between strains in any one species are highly significant and provide an experimental approach as to what constitutional factors predetermine caries-susceptibility. This appears to be one of the most hopeful approaches to the study of the dental caries problem. Do these strains have different abilities to utilize the food consumed? Do they have different nutritional requirements? Do they use the same basal essentials to form teeth of different composition? These are but the beginning of an almost endless series of questions which could be composed. Somewhere in the maze lies the answer to man's most prevalent disease.

CHOICE OF DIETS BY RATS

The behavior of experimental animals, given a choice of several diets, has received considerable attention in an effort to establish whether or not animals possess an ability to develop appetites commensurate with good nutrition (*Nutrition Reviews* **9,** *26 (1951)*). Recently, some British workers have reinvestigated the choice of diets by rats deficient in B-complex vitamins (D. E. Tribe and J. G. Gordon, *Brit. J. Nutrition* **7,** *197 (1953)*). The experiment was designed to find out if normal and deficient rats would

select the adequate diet when offered a choice of two diets identical in all respects except for their B-complex content. The effect of variation in position of the feeding vessel was also investigated.

The basal diet used (diet A) was the B-complex-deficient diet. It was of the purified type and contained casein, glucose, margarine, salts, corn starch, and a vitamin A and D supplement. The same diet supplemented with thiamine, pyridoxine, riboflavin, calcium pantothenate, niacin, inositol, and para-aminobenzoic acid (diet B) served as the B-vitamin-enriched diet. Twelve normal human subjects were unable to distinguish between the two diets by either smell or taste.

Five groups of rats, 100 g. in body weight, were used (4 to 6 animals per group). Group 1 was given a choice of diets A and B. On the first day the container for diet A was placed in pot A at the front of the cage and diet B in the rear of the cage in pot B. The second day and all succeeding days of the experiment the diet and pot positions were alternated. Group 2 was given diet A in two containers. When they showed B-vitamin deficiency symptoms, one of the containers was replaced by another containing diet B. Group 3 was treated as group 1, except that the position of the feed pots was kept the same and only the position of the diets was alternated. Group 4 was treated as group 1, except that here the positions of the diets remained the same while the pots were alternated. Group 5 was fed diet B only. The body weight increases of this group served as a positive control.

The animals of group 2 (B-vitamin-deficient) showed preference for diet B (B-vitamin-supplemented) and regained body weight. Seventy-four per cent of the food they ate was diet B. The rats of groups 1 and 3 consumed 56 and 62 per cent, respectively, of their total feed in the form of diet B. Thus, even normal rats were shown to possess a preference for the diet supplemented with B-vitamins, although the preference was not so great as that shown by the B-vitamin-deficient animals.

The results with group 4 were of particular interest since these animals showed no particular preference for either diet (50 per cent of total consumption as diet B). Since animals in groups 1 and 3 showed preference for diet B, this was an indication that position of feed in the cage was in favor of diet A.

These results confirm the observations of E. M. Scott and E. Quint (*J. Nutrition* **32,** *113, 285 (1946)*), who found that thiamine-, riboflavin-, and pyridoxine-deficient rats had appetites for the missing vitamins. Scott and Quint, however, did not find this to be true for normal rats.

The British workers also investigated the effect of different starches on the choice of diets by rats (D. E. Tribe and J. G. Gordon, *Brit. J. Nutrition* **7,** *202 (1953)*). The same basal diet (vitamin B-complex-deficient) was used and the substitution of potato or rice starch for the corn starch in the basal mixture was tested. The purpose of the study was to determine whether the rats would be able to select the diet on which they would synthesize the missing vitamins by refection. Based on the studies of S. K. Kon (*J. Hyg. (Cambridge)* **31,** *543 (1931)*) and F. Baker, H. Nasr, and F. Morrice (*J. Gen. Microbiol.* **3,** *xv (1949)*), the corn starch and rice starch diets were representative of diets on which refection does not occur whereas the potato starch diet was representative of one on which refection can occur. In preliminary trials it was established that the potato starch diet was distinctly less palatable than the other two diets.

The rats (100 g. body weight) were placed in four groups of 6 rats each which represented the following diet selection situations: group A, potato starch diet (unpalatable and refective) versus corn starch diet (palatable and not refective); group B, corn starch diet versus rice starch

diet (both palatable and not refective); group C, rice starch diet (palatable and not refective) versus corn starch diet plus B-vitamins (palatable); and group D, potato starch diet (unpalatable and refective) versus corn starch diet with B-vitamin supplement (palatable). The position of the feeds in the cages was alternated at irregular but frequent intervals to overcome feed position effects.

As might be expected on the basis of the results with B-vitamins, the rats of groups C and D preferred the palatable corn starch diet supplemented with B-vitamins over either the unpalatable diet that permitted refection or the palatable diet not permitting refection. When allowed a choice between two palatable diets, both deficient in B-vitamins but not permitting refection, (group B), the rats consumed equal quantities of each. All these animals failed to gain weight and were deficient in B-vitamins.

When allowed a choice of an unpalatable diet permitting refection and a palatable diet, not refective (group A), all the animals preferred the palatable diet, their body weights declined, and they died. In this case it was indicated that appetite was not conditioned by nutritional need.

It would be of interest to establish whether or not B-vitamin-deficient rats would prefer a palatable diet that permitted refection over a palatable diet that did not permit refection. Such a choice was not included in the experimental design that was tested by the British workers. The potato starch diet used by them was apparently not ideal for this type of investigation since the behavior of animals consuming the diet suggested that they were suffering from abdominal or digestive discomforts.

The results nevertheless are confirmatory of the view gained in earlier self-selection diet studies with rats. That is, even though B-vitamin-deficient rats possess a definite appetite for B-vitamins, they do not possess the uniform ability to select diets most conducive to optimum nutrition.

PREVENTION OF TOXIC EFFECTS OF GROWTH HORMONE

It is now widely agreed that there exists an anterior pituitary factor (or factors), other than corticotrophin (ACTH), which plays a significant role in carbohydrate metabolism. This factor has been called growth hormone or somatotrophin (STH). However, the term "growth" has become somewhat ambiguous since F. G. Young (*Biochem. J.* **39,** *515 (1945)*) proposed the theory that the "growth" effects of growth hormone might actually be mediated via insulin. Young's view has been developed by C. H. Best in a recent communication (*Ann. Int. Med.* **39,** *433 (1953)*). Moreover, M. S. Raben and V. W. Westermeyer (*Proc. Soc. Exp. Biol. Med.* **80,** *83 (1952)*) have reported separation of a pituitary preparation with growth-promoting activity but little, if any, diabetogenic effect. Evidence also has been presented which suggests that growth hormone may be the trophic hormone for the alpha cells of the pancreas and upper intestinal tract, and thus capable of stimulating increased production of so-called hyperglycemic factor (glucagon) (P. P. Foa, E. B. Magid, M. D. Glassman, and H. R. Weinstein, *Ibid.* **83,** *758 (1953)*).

Although the nature of this pituitary principle and its mechanism of action are far from clear, considerable information has accumulated about its effect on carbohydrate and protein metabolism. R. C. De Bodo and M. W. Sinkoff (*Ann. N. Y. Acad. Sci.* **57,** *23 (1953)*) have described extensive studies on the role of growth hormone in carbohydrate metabolism. These

investigators produced various hormonal deficiency states in dogs and studied a number of replacement therapies in order to ascertain the relative significance of each of the anterior pituitary hormone preparations under these circumstances. Trained, unanesthetized adrenalectomized, adrenalectomized-gonadectomized, and adrenalectomized-hypophysectomized dogs served as experimental subjects.

It was shown first that the adrenalectomized dogs were far less sensitive to insulin than the adrenalectomized-hypophysectomized animals, in which a striking insulin hypersensitivity was noted. The differences in insulin sensitivity occurred whether the adrenalectomized dogs were maintained on desoxycorticosterone acetate or not. These findings were interpreted as providing further support for the view that some anterior pituitary factor(s) other than ACTH does indeed have a role in normal carbohydrate metabolism. Studies on adrenalectomized-gonadectomized dogs disclosed that the gonadotrophins cannot be implicated as influencing the regulation of carbohydrate metabolism inasmuch as the adrenalectomized and the adrenalectomized-gonadectomized dogs had similar responses to insulin and intravenous glucose.

When growth hormone was administered to hypophysectomized dogs insulin hypersensitivity was completely abolished along with the secondary hypoglycemia following glucose administration. However, such treatment also produced diabetes and insulin resistance. In addition, toxic manifestations such as anorexia, vomiting and weakness were observed, sometimes followed by death. Lower doses of growth hormone over shorter intervals of time were capable of abolishing the insulin sensitivity of the hypophysectomized dogs without altering glucose tolerance or producing insulin resistance. Yet, even during minimal dose regimens, 50 per cent of the hypophysectomized dogs tended to develop diabetes. Thus, it was of interest that when hypophysectomized dogs were treated with ACTH or cortisone alone their carbohydrate metabolism appeared to revert to normal. For example, continued administration of the cortical steroids abolished the insulin hypersensitivity of the hypophysectomized dogs without production of insulin resistance. The normal response to intravenous epinephrine was restored and secondary hypoglycemia following glucose tolerance test no longer occurred. When growth hormone administration was then superimposed upon adrenocortical steroid therapy, insulin resistance and diabetes were not observed. Indeed, neither a summation nor a potentiation of the individual effects of these two blood sugar-raising agents took place.

As an incidental finding, it was shown that only growth hormone was capable of raising the impaired renal function of the hypophysectomized animal to normal.

From their experiments, the authors concluded that growth hormone does not require the adrenal or sex steroids in order to manifest its anti-insulin and diabetogenic actions. However, to produce its effects *without* associated diabetes or toxicity, it would appear that growth hormone does need the presence of the adrenocortical steroids.

Previous work has shown that growth hormone probably influences carbohydrate metabolism by interfering with peripheral utilization of glucose. However, purified growth hormone decreases glucose uptake by the isolated rat diaphragm only if the hormone is injected into the living animal. Growth hormone is inactive if added directly to the diaphragm *in vitro* (C. R. Park and W. H. Daughaday, *Fed. Proc.* **9**, *212 (1950)*). Accordingly, it is not clear whether growth hormone per se acts on carbohydrate utilization or whether it acts via another substance such as hyperglycemic factor.

From the clinical standpoint perhaps the

most significant aspect of the work of De Bodo and Sinkoff has been their demonstration of the apparent protective effect of adrenocortical steroids against the toxic effects of growth hormone. One may wonder whether cortisone or hydrocortisone might not be of value in the treatment of active acromegaly. Conversely, growth hormone might protect against some of the undesirable effects of the adrenocortical steroids.

Since growth hormone in the presence of insulin has a potent protein anabolic effect, it must be considered to be of potential use in the treatment of certain nutritional disorders. Hence, the finding of De Bodo and Sinkoff that the toxic effects of growth hormone may be mitigated or prevented by concomitant administration of adrenocortical steroids may prove to be of considerable practical importance to the clinical nutritionist.

ENDOCRINE FUNCTION OF THE PANCREAS IN OBESE MICE

The development of a strain of obese diabetic mice has been discussed (*Nutrition Reviews* **10,** *303* (*1952*)). The similarities between the diabetes of these mice and the diabetes which occurs in obese middle-aged human beings makes this syndrome of general interest. K. Guggenheim and J. Mayer (*J. Biol. Chem.* **198,** *259* (*1952*)) have attributed the metabolic disorder to a defect in acetate oxidation. When C^{14} carboxyl-labeled acetate was injected into fasting obese mice one-third less of the carbon label appeared in the expired carbon dioxide of the obese mice than in that of nonobese mice similarly injected. It was also found that more of the labeled acetate was incorporated into fatty acids by the obese mice. From these observations it was concluded that there was a block in acetate oxidation. Because acetate was not normally oxidized, more was made available for fatty acid synthesis. As a corollary to this theory, a decreased capacity for lipolysis could be postulated.

The original histologic description of the endocrine glands of obese mice demonstrated islet cell hyperplasia. J. Mayer, S. B. Andrus, and D. J. Silides (*Endocrinology* **53,** *572* (*1953*)) have made a more thorough study of islet cell function. The beta cells of obese mice were found to be distinctly deficient in granules, the blood supply to these islets was more prominent, and a clear zone was often found between capillaries and adjacent beta cells. Reference was made to joint unpublished studies with G. A. Wrenshell of the Banting and Best Institute which indicated that the pancreases of mature obese-hyperglycemic mice contained several times more insulin than the pancreases of nonobese mice. This finding apparently is an exception to the usual assumption of a correlation between beta granules and insulin content.

The effect of agents injurious to alpha cells was investigated. The first of these agents, diethyldithiocarbamate (DEDTC) has been found by I. Kadota and O. Midorikawa (*J. Lab. Clin. Med.* **38,** *671* (*1951*)) to produce lesions in the alpha cells of the rabbit pancreas. When this drug was given to normal mice in subcutaneous doses of 0.2, 1 and 2 mg. an immediate rise in blood sugar occurred which was followed by a return to high normal levels. After a dose of 15 mg. hyperglycemia persisted for ten to thirteen days. The response of the obese mice was different. Twenty-four hours after similar doses of diethyldithiocarbamate, the blood sugar level had fallen from about 200 mg. per cent to 130 to 140 mg. per cent—a high normal range. A slow rise in blood glucose levels occurred during the four to eight days after treatment. By fourteen

days the pretreatment hyperglycemia was restored. Obese mice lost 4 to 6 g. during this period whereas nonobese mice gained an average of 7 g. The fall in blood sugar and the weight loss could not be attributed to inanition.

An alteration in the response to growth hormone occurred after DEDTC was administered. Untreated obese animals respond to growth hormone with marked hyperglycemia. In DEDTC-treated animals no change in blood sugar occurred.

Insulin resistance, one of the cardinal disturbances of this syndrome, was decreased by DEDTC. Whereas 20 units of regular insulin had little effect on the blood sugar of noninjected obese mice, the injection of 10 units of insulin into 10 obese mice treated with 15 mg. of DEDTC produced hypoglycemic convulsions.

In view of the definite metabolic alterations which occurred in the obese mice after DEDTC administration, the histologic changes in the pancreas are disappointing. No alterations in the alpha cells were found in either the obese or nonobese groups. In the obese group, however, striking *increase* in beta granules occurred. Lesions in other organs were not found.

The second agent known to affect alpha cell function is cobaltous chloride. Degenerative lesions of the alpha cells had been previously produced by this agent in the guinea pig (E. Van Campenhout and G. Cornelis, *Compt. rend. Soc. de biol.* **145,** *933 (1951)*) and the rabbit (M. G. Goldner, B. W. Volk, and S. S. Lazarus, *Metabolism* **1,** *544 (1952)*). Subcutaneous doses of 2.5 to 10 mg. of cobaltous chloride proved too toxic. A dose of 1.25 mg., however, was tolerated by the obese mice. As in the DEDTC-treated animals, an immediate hyperglycemia was followed by subsequent fall in blood sugar so that the levels were lower after four to seven days than they were before treatment. The changes were much less definite than those which occurred after DEDTC administration. Again no histologic lesions in the alpha cells occurred.

The authors have interpreted the metabolic and morphologic data as suggesting that the pancreas of obese mice secretes an excess of a hyperglycemia-producing "anti-insulin" fraction, the release of which is promoted by growth hormone and blocked temporarily by the administration of DEDTC. Independent experimental support for the view that growth hormone may act on the pancreas to cause the release of hyperglycemic factor has been provided by the studies of portal vein blood in growth hormone-treated dogs (J. Bornstein, E. Reid, and F. G. Young, *Nature* **168,** *903 (1951)*). The failure to find histologic evidence of alpha cell damage after DEDTC administration throws in doubt the site of action of the drug. From the evidence presented the islets may only be secondarily affected. Because excessive production of hyperglycemic factor in this syndrome has been proposed, the effect of chronic administration of hyperglycemic factor free of insulin on obese and nonobese mice would be important.

METABOLISM OF GLYCEROL

Glycerol has long been a neglected stepchild of biochemistry. Although it is a necessary component of triglycerides and hence a compound of universal importance, it has been all but forgotten in studies of lipid physiology. It is a welcome sign that two excellent studies on glycerol, one dealing with its synthesis and one with its catabolism, have appeared recently.

R. Reiser and M. C. Williams (*J. Biol. Chem.* **202,** *815 (1953)*) have studied the synthesis of glycerol in the course of fat

absorption. Previous work from their laboratory (Reiser, M. J. Bryson, M. J. Carr, and K. A. Kuiken, *Ibid.* **194,** *131 (1952)*) had demonstrated that C^{14}-labeled glycerol, completely hydrolyzed from glycerides during digestion, was not utilized for the synthesis of the triglycerides isolated from the lymph. This unexpected finding suggested that glycerol per se was probably not the precursor of the glycerol found in glycerides. Since, however, absorbed fatty acids appear in the lymph as triglycerides, it remained to account for the origin of the glycerol required to form lymph fat and body fat from the considerable amounts of dietary fat which may be consumed and digested in the span of a few hours. As it is known that two compounds closely related to glycerol, dihydroxyacetone and glyceraldehyde, occur normally in carbohydrate metabolism, it appeared logical to examine the possibility that they are precursors of glycerol. Both molecules differ from glycerol simply through the one-step oxidation of one carbon atom; conversely the reduction of an aldehyde to a primary alcohol and of a ketone to a secondary alcohol are frequently effected in the body; finally, the fact that the degradation of every molecule of glucose gives rise to one molecule of dihydroxyacetone and one of glyceraldehyde made possible a solution to the "logistic" problem of rapidly synthesizing the large amounts of glycerol needed for fat absorption.

It appeared possible, therefore, that dihydroxyacetone and glyceraldehyde were precursors of glyceride glycerol, forming monoesters and diesters with fatty acids, then being reduced to glycerol and finally forming the triglycerides. Reiser and Williams tested this hypothesis by feeding rats the monopalmitic acid ester of dihydroxyacetone labeled in both the acetone (C^{14}-alcohol) and fatty acid (C^{14}-carboxyl) moieties and examining the lymph for labeled glycerol. This double labeling would reveal at least two important points. The relative amounts of labeled glycerol and labeled dihydroxyacetone in thoracic duct lymph glycerides should be a measure of the extent to which dihydroxyacetone was converted to glycerol. The difference in percentages of recovery in the lymph of total labeled alcohol (whether dihydroxyacetone or glycerol) and of labeled fatty acid should be a measure of the degree of hydrolysis of the absorbed palmitoxyhydroxyacetone since it represents the relative amount of alcohol lost. Reiser and Williams also verified the old observation (O. Frank, *Z. f. Biol.* **36,** *568 (1898)*) that ingested monoglycerides did not appear in the lymph by feeding rats cottonseed oil monoglyceride.

Analysis of the lymph fat demonstrated that injected monoglycerides were 73 per cent hydrolyzed when fed as such. This figure is higher than the proportion of 25 to 45 per cent hydrolysis found for triglycerides (Reiser *et al.*, *loc. cit.*). If, however, one reflects that in the absence of free fatty acids three molecules of monoglycerides are required to form one of triglycerides, a minimum hydrolysis of 66.7 per cent of monoglycerides becomes obligatory.

When palmitoxyhydroxyacetone rather than monoglyceride was fed, the proportion hydrolyzed was also of the order of 73 per cent. The fatty acid esters of dihydroxyacetone did not appear in the lymph unchanged, but were reduced and converted to triglycerides. The authors conclude that esterification of dihydroxyacetone with fatty acid and subsequent reduction and esterification may be the normal mechanism of triglyceride synthesis during fatty acid absorption. An alternative solution would be hydrolysis, reduction to glycerol and esterification, a possibility which does not fit with observed proportions of esters hydrolyzed. The evidence presented in this study led Reiser and Williams to hypothesize further that the hydrolysis of monoglycerides and synthesis of triglycerides takes place in the intestinal mucosa, or, at least, after absorption. This point of view is also strengthened by the contrast of the resistance of

monoglycerides to the action of intestinal lipase (B. Borgström, *Acta physiol. Scandinav.* **25,** *328 (1952)*) and of the ready absorption of monoglycerides (Reiser *et al.*, *loc. cit.*).

In a study dealing with the degradation by liver slices from fasted rats of glycerol-1-C^{14}, C.-T. Teng and co-workers (*J. Biol. Chem.* **202,** *705 (1953)*) found that about 86 per cent of the glycerol metabolized was accounted for as follows: 71 per cent as glycogen and glucose, 5 per cent as carbon dioxide, 3 per cent as lactate. The other fractions, including lipids and proteins, accounted for the remainder.

Glycerol was also shown to interact with the metabolism of added pyruvate by decreasing its transformation to glycogen and to carbon dioxide and doubling its reduction to lactate. While the over-all physiologic interpretation of this study is more obscure than the report of Reiser and Williams, it constitutes a step in the much needed attack on the catabolism of glycerol.

ASCORBIC ACID AND ACTH IN EXPERIMENTAL WOUNDS

Impairment of wound healing in scorbutic sailors was described centuries ago. In the intervening years, these observations have been repeatedly confirmed in animals and patients with experimental or clinical scurvy. About twenty-five years ago, S. B. Wolbach and P. R. Howe (*Arch. Path. Lab. Med.* **1,** *1 (1926)*) demonstrated by the then available histologic technics an apparent delay in the appearance of ground substance and in the maturation of collagen in experimental wounds in scorbutic guinea pigs. Fibroplasia was not impaired, but maturation of the fibroblasts was. More recently, marked effects of certain of the adrenal cortical hormones on connective tissue have been recognized. Under certain conditions of ACTH or cortisone administration, there is a delay in the deposition of fibroblasts, ground substance and collagen in healing wounds. J. A. Schilling and his associates (*Surg. Gynec. Obstet.* **97,** *434 (1953)*) have recently reported a study undertaken to determine whether there may be a relationship between ascorbic acid and ACTH in wound healing.

Male guinea pigs about 400 g. in weight were studied. Normal control animals were fed a standard commercial diet fortified with 20 mg. of ascorbic acid per 100 g. of the diet. Other animals were made scorbutic by being maintained on an ascorbic acid-deficient diet. Six groups of animals were studied: first, a normal group of animals; second, a scorbutic group; third, a group of animals which received in addition to the standard "adequate" diet supplementary ascorbic acid, 10 or 50 mg. daily, either by mouth or subcutaneous injection; fourth, a group of animals on the standard "adequate" diet plus 0.005 g. of ACTH intramuscularly twice a day; fifth, a group of scorbutic animals which received ACTH in similar dosage; and finally a sixth group of animals which while on the standard "adequate" diet received both ACTH and ascorbic acid.

In one series of experiments involving 90 animals, fibroplasia and collagen production were followed by estimating the amount of growth in and around a subcutaneously implanted hollow tantalum mesh sphere. In addition to study of the gross appearance of the area in and about the sphere, histologic observations were made, using routinely hematoxylin and eosin stain and Mallory's aniline blue stain for collagen. Further, the behavior in tissue cultures of fibroblasts obtained from the "wounded" area was observed. In the scorbutic animals, fibrosis was decreased and very little ground substance or collagen was laid down. Those animals receiving ACTH, both scorbutic

and nonscorbutic, demonstrated during the first ten or twelve days following implantation of the tantalum sphere more hemorrhagic reactions than similar animals not receiving ACTH. Later, no differences were apparent. No delay in fibroplasia or ground substance and collagen formation specifically attributable to ACTH administration was observed. In preliminary observations, it appeared that the fibroblasts from animals receiving ACTH did not grow so vigorously in tissue culture as fibroblasts from normal controls.

In a second series of experiments, a standard wound was made in the dorsum of the animals by excising a portion of skin and subcutaneous tissue. The excised area measured 2.5 cm. in diameter. The rate of wound closure was followed by a photographic technic. No histologic observations were made. Fifteen nonscorbutic animals were used in this series. Administration of ACTH or ascorbic acid did not affect the rate of the wound closure. No specific statement is made whether an additional 10 or 50 mg. of ascorbic acid daily were given.

In a third series of animals, three linear wounds, each 3 cm. in length, were made on the dorsum of each guinea pig. The incisions extended through the panniculus carnosus. These wounds were immediately sutured with fine tantalum wire. After nine days, the sutures were removed, the animals were killed, and the tensile strength of the wounds was measured. The tensile strength of the scorbutic animals was low, about 40 per cent of normal. ACTH given to scorbutic animals had no effect on wound strength. Similarly, no statistically significant differences were observed among 24 nonscorbutic animals on the standard "adequate" diet and receiving 10 or 50 mg. of additional ascorbic acid daily with or without ACTH.

In some animals the ascorbic acid concentration in the blood serum and tissue fluids of the wounds was measured. The administration of ACTH had no apparent effect on the concentrations of ascorbic acid in either the serum or tissue fluid in nonscorbutic animals receiving or not receiving supplemental ascorbic acid. Among the scorbutic animals, a higher concentration of ascorbic acid was noted in the tissue fluid as compared with the blood serum. No data on the effect of ACTH on the ascorbic acid levels in the scorbutic animals are presented. Schilling and his associates observed a day-to-day fluctuation in the tissue fluid concentration of ascorbic acid which seemed to follow a pattern independent of the administration of ascorbic acid or ACTH or the blood serum levels of ascorbic acid. It is suggested by the authors that these variations in ascorbic acid concentration in the wound fluid may be significant and may represent different phases or velocities of the reparative processes. However, only average values of ascorbic acid concentration in the tissue fluid are presented. Whether the fluctuations are statistically significant is not definitely stated.

The authors interpret their observations to indicate that there is no significant relation between ACTH and ascorbic acid in wound healing under the experimental conditions studied by them. They also make the statement that in another group of experiments conducted by them in which cortisone was used instead of ACTH, no significant effect of cortisone on wound healing was observed. However, the amount of cortisone used is not stated.

The authors refer in their discussion to an abstract of a study carried out by C. L. Pirani, R. C. Stepto, and C. F. Consolazio (*Fed. Proc.* **11,** *423 (1952)*). Guinea pigs were maintained on "scorbutic," "normal" and "fortified" ascorbic acid intakes (0.0, 2.5 and 25.0 mg. ascorbic acid per day). On the tenth day of these regimens, laparotomy wounds were made. Thereafter, half of the animals of each group were injected with ACTH (6 mg. per

day) and half with saline. Postoperatively, on various days, the animals were killed and the wounds were studied histologically. In the saline group, healing in the "scorbutic" animals was impaired, while the "fortified" guinea pigs, especially on the fifth and tenth postoperative days, showed better healing than the "normal" animals. In the ACTH group, a moderate inhibition of fibroblastic proliferation and collagen formation was observed in the "normal" animals on the fifth postoperative day but was less apparent later. ACTH-induced effects were minimal in the wounds of both "fortified" and "scorbutic" animals.

The findings of Pirani, Stepto and Consolazio are thus somewhat different from those of Schilling and his associates. The latter group found no evidence of beneficial effects on wound healing of 10 to 50 mg. daily of supplemental ascorbic acid over that provided by the standard "adequate" diet. Pirani's group, however, observed that the wounds of guinea pigs receiving 25 mg. of ascorbic acid daily healed "better" than those of animals receiving 2.5 mg. (their "normal" intake) of ascorbic acid daily. Similarly, Pirani and co-workers observed a detrimental effect of ACTH on wound healing in guinea pigs on a "normal" intake of ascorbic acid, while Schilling and his co-workers did not. However, it is not clear whether the normal control animals in the latter study received about the same amount of ascorbic acid daily, that is, about 2.5 mg., as the comparable group in the former investigation. Further, the effect of total food intake cannot be evaluated since in neither study are the data concerning total food intake given nor is it stated whether paired-feeding was carried out. Finally, different types of wounds were used in the two studies. Both groups of investigators interpret their findings as indicating that the role of ascorbic acid in wound healing is a direct one and not mediated through the adrenal cortex.

Available data suggest that ACTH in the dosages employed clinically has relatively little effect on wound healing. On the other hand, there is evidence that cortisone may inhibit wound healing. However, it should be pointed out that the delay in wound healing seen following cortisone administration is distinctly different from that seen in scurvy. In cortisone-treated animals, the delay in wound healing is primarily a delay in fibroplasia; once fibroblasts are formed, formation of ground substance and collagen proceeds. These events, then, are similar to those seen in protein-deficient animals. On the other hand, there is no delay in fibroplasia in scurvy. In the scorbutic individual fibroplasia proceeds at a normal or increased rate, but maturation of the fibroblasts is delayed. In addition, there is an apparent delay in the laying down of ground substance and collagen. Recent studies indicate that the defect in scurvy may not only be a quantitative one, but that qualitative changes in the ground substance and collagen may also occur (*Nutrition Reviews* **10,** *124 (1952)*; **11,** *82 (1953)*).

NOTES

Molybdenum Content of Foods

It has been established that molybdenum is an essential trace element in animal nutrition. This element is necessary as a dietary constituent because its presence favors the elaboration of an important oxidative enzyme, namely xanthine oxidase (*Nutrition Reviews* **11,** *250* (*1953*)). The activity of this enzyme is very sensitive to nutritional disturbances, not only with regard to molybdenum, but also with regard to dietary protein. The activity of this enzyme in rat liver can in fact serve as a method for determining the nutritive value of dietary protein (*Ibid.* **11,** *23* (*1953*)).

In order to establish the value of various foods as sources of molybdenum, workers at the State University Medical College, Syracuse, New York, have recently analyzed a variety of foods for total molybdenum (chemical assay) and also for their ability to stimulate xanthine oxidase activity in rat intestine (biologic assay) (W. W. Westerfeld and D. A. Richert, *J. Nutrition* **51,** *85* (*1953*)).

Weanling rats were depleted of xanthine oxidase activity by feeding an adequate protein diet low in molybdenum for six to ten days. The depleted animals were then fed the test diets containing molybdenum from various natural food sources for a period of seven days. The test diets were identical with the depletion diet, except that the food under test replaced an equal amount of glucose in the basal mixture. The animals were then killed and their intestines were analyzed for xanthine oxidase activity.

The increase in xanthine oxidase activity observed in the rat intestines, as a result of supplying various test foods in the diet, in general correlated quite well with the total molybdenum content of the test foods. From 50 to 100 per cent of the dietary molybdenum was usually available for the stimulation of enzyme activity.

Foods considered to be good sources of molybdenum and containing 0.6 parts per million (p.p.m.) of available molybdenum (promoting enzyme activity) on a dry weight basis were: legumes, cereal grains, some dark green, leafy vegetables, and animal organs (liver, kidney, and spleen). Fruits, berries, and most root or stem vegetables were relatively poor sources of molybdenum, containing less than 0.1 p.p.m. Brewers' yeasts were found to be good sources of molybdenum, whereas bakers' yeasts were poor sources even though the growth mediums in both cases were not rich in molybdenum.

Recent Nutrition Books

Nutrition in Health and Disease. Lenna F. Cooper, Edith M. Barber, Helen S. Mitchell, and Henderika J. Rynbergen. 12th edition. J. B. Lippincott Company, Philadelphia, Penna., 1953. Pp. 727.

Plant Proteins in Child Feeding. R. F. A. Dean. Medical Research Council Special Report Series No. 279. Published by Her Majesty's Stationery Office, London, 1953. Pp. 163. Price: $2.25.

Present Knowledge in Nutrition. Prepared from articles published in the journal *Nutrition Reviews* by its Editorial Staff and Advisory Board, 1953. Copies may be obtained from The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. Pp. 122. Price: $2.00. (Supplement included.)

Present Problems in Nutrition Research. F. Verzar, Editor. Published by Verlag Birkhauser, Basel-Stuttgart, 1953. Pp. 309.

Biochemistry and Physiology of Nutrition. Volume I. Edited by Geoffrey H. Bourne and George W. Kidder. Academic Press, Inc., New York, N. Y., 1953. Pp. 569. Price: $13.00.

The Limits of the Earth. Fairfield Osborn. Little, Brown and Company, 1953. Pp. 238. Price: $3.50.

"It is apparent from these studies that intestinal absorption of vitamin B_{12} after total gastrectomy is as poor as in pernicious anemia and is corrected by the concurrent administration of gastric juice. This finding may be interpreted as indicating that the gastric mucosa is almost the sole source of intrinsic factor." (*Nutrition Reviews* **12,** *4* (*1954*), Vitamin B_{12} Absorption After Gastrectomy.)

"Serum amylase and esterase determinations were made to ascertain the influence of frank kwashiorkor on digestive enzymes. All but one of 14 children studied had serum amylase values below 50 Somogyi units. These increased to about 100 units during twenty-eight days of treatment. Serum esterase values were likewise low initially." (*Nutrition Reviews* **12,** *5* (*1954*), Serum Chemistry in Kwashiorkor.)

"The repeated injection of ACTH or cortisone was followed by definite changes in the concentration of sodium in human saliva. The sodium level of resting and stimulated saliva declined within four hours after the beginning of injections in each of the 5 subjects." (*Nutrition Reviews* **12,** *7* (*1954*), Dietary Influences on the Composition of the Saliva.)

"Another point of great interest is the variety of signs and symptoms which may occur in vitamin B_6 deficiency induced by this means. The chief and most common clinical manifestations were those of anorexia, nausea, lethargy, seborrheic dermatitis, cheilosis, conjunctivitis, glossitis, pellagra-like dermatitis, and polyneuritis." (*Nutrition Reviews* **12,** *10* (*1954*), Vitamin B_6 Deficiency in Man.)

"The child with kwashiorkor is not an incidental, or a seasonal or regional finding. He is found everywhere and at every season. Such children have been known for many years. Nutritional advances in recent years have given us a clearer insight into the etiology and the possibilities for efficient therapy and prevention of this disease." (*Nutrition Reviews* **12,** *33* (*1954*), Infantile Malnutrition in Indonesia.)

"The experimental demonstration that body weight is the most important variable which must be considered in determining the energy needs deriving from expenditure of physical energy should be useful in determining calorie requirements of population groups in industry and in a variety of other activities." (*Nutrition Reviews* **12,** *36* (*1954*), The Energy Cost of Muscular Exercise.)

"These studies suggest that the amount of fat in the diet largely, but not completely, influences fecal lipid excretion in man. Thus, since lipids continue to be excreted during the ingestion of a lipid-free diet such as this, it is clear that there is a certain portion of the fecal lipid which is probably derived from such sources as secretion, desquamation, or bacterial synthesis." (*Nutrition Reviews* **12,** *37* (*1954*), Fecal and Plasma Lipids on Fat-Free Diets.)

"On the basis of these results the apparent rapid assimilation of fructose in normal man can be attributed to two factors: (1) a rapid rate of phosphorylation of fructose, particularly in the liver, with eventual conversion to lactate, pyruvate, glucose, glycogen and other products; (2) a much larger volume of distribution in body water than exists for glucose." (*Nutrition Reviews* **12,** *39* (*1954*), The Fate of Intravenous Fructose.)

"The most interesting and perhaps most significant findings which are recorded in this report pertain to the morphologic examination of the liver biopsy specimens. It is demonstrated that fat accumulates in both intracellular and extracellular sites." (*Nutrition Reviews* **12,** *41* (*1954*), The Acute Fatty Liver in Man.)